HONOURS PHYSIOLOGY 1975-1976

DISSERTATION

ECTOPIC HORMONE PRODUCTION BY HUMAN TUMOURS

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CONTENTS

ABBREVIATIONS

ACTH - ADRENOCORTICOTROPHIC HORMONE

ADH - ANTIDIURETIC HORMONE

PTH - PARATHYROID HORMONE

HGH - HUMAN GROWTH HORMONE

HCG - HUMAN CHORIONIC GONADOTROPHIN

5HT - 5-HYDROXYTRYPTAMINE

A. INTRODUCTION

The recognition that certain clinical syndromes of endocrine hyperfunction can be due to ectopic polypeptide hormone production by tumours has been among the major developments in endocrinology in the last decade. A hormone is defined as ectopic when it is secreted by a tumour derived from a tissue not normally engaged in the production of that hormone, (41) e.g. an oat cell carcinoma of lung secreting ACTH (93) or an islet cell carcinoma of pancreas secreting ADH (40).

It is interesting that neoplastic cells can acquire the capacity to produce specialized polypeptides such as hormones and this phenomenon would appear to reflect significant alterations of genetic regulation (3,4). Ectopic hormone production by tumours is a pathological event which raises many important questions concerning cell differentiation and its control.

B. EVIDENCE FOR HORMONE PRODUCTION BY TUMOURS

Despite numerous reports of ectopic hormone producing tumours, few have given conclusive evidence for ectopic production. Indeed, one recent critical evaluation (8) of reports of tumours associated with Cushing's syndrome i.e. ectopic ACTH secretion, rejected approximately 25% of these because of inadequate evidence of ACTH secretion by the tumours or misidentification of the tumour's histological type. To avoid such inaccuracy, several authors have suggested criteria for the ectopic production of a hormone by a tumour (68,77,78). These criteria are based on the existing evidence for ectopic hormone production, and are as follows:-

1. Association of a tumour with an endocrine excess syndrome

It was from such associations (72,73) that the concept of ectopic hormones emerged, but now this is nothing more than a starting point for diagnosis. High plasma levels in a tumour-bearing patient need not be result of ectopic production as there could be a coexisting pathological condition e.g. idiopathic Cushing's syndrome.

2. Demonstration of the presence of hormone in the tumour tissue

Tumour cells, suspected of being hormone-producing, have been shown to have concentrations of hormone in their cytoplasm and this can be demonstrated in two ways:-

i) Biochemical extraction of the hormone from the tumour. The extract is then tested by bioassay (57).

ii) Immunofluorescent techniques. This involves using fluorescent-labelled antisera for the suspected hormone (29,41), and requires isolated and purefied natural hormones for production of the antisera.

Positive results, using either of these methods, do not

necessarily prove that the tumour has synthesized that hormone (40). Some tumours seem capable of selectively extracting hormones from the circulation (35) and this phenomenon has also been reported for tuberculous lesions which had high concentrations of ADH present (74).

3. Demonstration of an arterio-venous concentration gradient for the hormone across the tumour bed

Arterio-venous gradients have been reported for a variety of hormones, namely ACTH (10,71), PTH (14,17,39), gonadotrophins (6,23), and HGH (78), and these findings offer more convincing evidence for hormone secretion by tumours. Difficulties can arise, however, when tumours have a dual blood supply e.g. in the lung (40).

4. Lack of suppression of secretion due to autonomous production.

Despite isolated pieces of evidence for periodic hormonogenesis in tumours (9) and for stimulation of production by normal releasing factors (11,36), most ectopic hormone producing tumours appear to secrete hormone continuously and are not subject to the feedback control of normal endocrines (10,29,35,41,71). Autnnomous production of hormone by the tumour can be demonstrated by the use of substances ~~which~~ which interfere with the normal endocrine systems e.g. metyrapone inhibits the 11-hydroxylation stage in the synthesis of cortisol and, in the normal subject, leads to increased plasma ACTH levels. In cases where an ectopic ACTH secreting tumour is present, the plasma ACTH remains unaltered after metyrapone (35). Similarly, hydrocortisone or dexamethasone administration fails to suppress ACTH levels (10,29,71).

The most striking finding in this context is the marked atrophy of the endocrine gland normally associated with a hormone which was present in very high levels before death (69).

5. In vitro synthesis and release of hormone

Perhaps the most convincing evidence for ectopic hormone production comes from cell culture work in which tumour cells have been shown to synthesize hormones. Cells cultured in vitro from an anaplastic (probably oat-cell) lung carcinoma have been shown to incorporate radioactively labelled leucine into an HGH-like substance (32) (the radioactivity appeared in a fraction which separated out alongside standard HGH in paper chromatography and gel filtration). HGH was also detected by radioimmunoassay in the supernatant and was found to accumulate over a period of time. Similar results have been obtained for bronchogenic carcinomas synthesizing ADH (28), ACTH (36) and HCG (6). The latter study, however, merely demonstrated an increase in radioimmunoassayable hormone in the supernatant. This does not necessarily indicate de novo synthesis as hormone could be stored in the cells and be released on cell death.

From the above discussion it can be readily appreciated that it is difficult to produce direct evidence of hormone synthesis by a tumour, althouhg the accumulation of evidence from many sources does render ectopic hormone synthesis by tumours an entity. Since, however, some explanations of the ectopic hormone phenomenon rely on knowing the exact cells of origin (8) , it is imperative that accurate evidence be obtained. This is particularly difficult when a tumour contains several cell types.

C. NATURE OF THE HORMONES SECRETED BY TUMOURS

Crucial to the consideration of the mechanism of ectopic hormone production is the question of whether the hormones secreted are identical to the normal ones. As yet, no amino-acid sequence has been obtained for an ectopic hormone but information regarding their structure has come from biochemical, radioimmunoassay and chromatographic techniques. (41,42) These preliminary characterizations suggest that the hormones are indeed similar to the native ones although there is some contradictory evidence. Ectopic ACTH has been studied intensively (29,36,41,51,52,53,97) and it appears that tumours tend to secrete a 'big' form of ACTH (29,36,41,97) because the material extracted with ACTH-like activity has the chromatographic mobility of a larger molecule (estimated m. w. of 7000 daltons c. f. m.w. of normal pituitary ACTH of 4500 daltons). A credible explanation for this (31) is that it represents a precursor form of ACTH and that tumours may lack the enzyme(s) necessary to convert it to the normal circulating form. This hypothesis is supported by the finding of 'big' ACTH in the normal pituitary (97).

It is interesting to note that two studies (29,35) have found that, in biopsy or autopsy samples of lung carcinomas from patients with no evidence of ectopic ACTH syndrome, there was a significant concentration of 'big' ACTH in the tumour c.f. adjacent lung tissue. 'Big' ACTH has a very low biological potency ($4\frac{1}{2}\%$ of normal ACTH) and it is possible that ectopic synthesis of ACTH is more common than imagined but goes unnoticed. Moreover, it is conceivable tha t the only cases tested are ones in which the hormone produced is similar enough to the normal one to produce a clinical syndrome while other tumours may be synthesizing polypeptides which partially resemble the native hormone but whose hormonal potency is low i.e. diagnostic procedures may be biasing results.

D. DISCUSSION OF THEORIES ON MECHANISM OF ECTOPIC HORMONE SYNTHESIS BY TUMOURS

Since ectopic hormone production by tumours was recognized as an entity, several theories have been advanced to explain the mechanism involved. These are as follows:-

i) Sponge hypothesis

Unger (81) thought it highly unlikely that disorganized cells such as neoplastic cells could suddenly acquire the specialized synthetic functions of endocrine cells. Instead, he introduced the 'sponge' hypothesis which stated that large quantities of hormone accumulated in the tumour mass as a resultof increased hormone uptake and/or decreased degradation by neoplastic cells. If, then, the patient survived long enough to develop large hormone-laden tumour masses, accelerated brea kdown of malignant cells in rapidly enlarging tumours could result in an unregulated release of large quantities of hormone and the appearance of hormone excess. Support for this theory comes from the finding (30) that neoplastic cells are capable of taking up fluorescently-labelled proteins directly. This process is distinct from the known capacity of tumour cells, and all rapidly growing tissues, to sequestrate amino-acids from the common metabolic pool. In addition to this, the previously mentioned reports of concentration of hormone (ADH) in tuberculous lesions (74,83) could be as a result of this 'sponge' effect.

Unger's hypothesis, however, is unacceptable for the following reasons:-

a) the concentrations of hormone in the tumour cell cytoplasm (see B.2) were approximately 4000-6000 X the plasma levels. It is difficult to envisage such a concentration occurring by a 'sponge'

process.

b) certain types of tumours are associated with particular hormones (8). This non-random association is not likely to occur if the mechanism merely involves 'trapping' of the hormone in tumour cells.

c) the evidence for hormone synthesis by cell cultures from tumours (6,28,32) and, in particular, the demonstration of HCG production in males (28) renders the 'sponge' hypothesis unlikely.

ii) Random genetic de repression

From experiments such as those in which nuclei have been transplanted from differentiated adultcells to enucleated ova (in frogs) and normal development has ensued (3,27,33), it is known that all cells possess and retain the genetic information for the synthesis of all proteins. Differentiation is thought to involve progressive repression of genes and, indeed, it has been shown that there is a reduction in DNA template activity in the course of cell differentiation (47) (the technique involved the use of a histochemical method for highlighting active DNA template along with high resolution electron microscopy)

The random derepression hypothesis (26) postulates that the genetic derepression caused by neoplasia (4,75) can lead to the production of any polypeptide or protein depending on which part of the genome is derepressed. Ectopic hormone synthesis is merely one example of this effect.

As yet, examples of ectopic polypeptide hormones only have been obtained. Since steroid hormone production requires the presence of several enzymes, it is unlikely that these would all appear as a result of random derepression (12,30). This would seem to support the hypothesis but random derepression, However, fails to explain why certain tumours e.g. carcinomas of breast, colon

and prostate, are very rarely associated with ectopic hormones (49) whereas other tumours are frequently involved (8) e.g. islet-cell carcinomas, medullary carcinoma of thyroid, oat-cell carcinoma of lung and carcinoid tumours.

iii) Selective genetic derepression

As mentioned in the previous section, neoplasia involves dedifferentiation of cells with the acquisition of an earlier competence (4,41,48,77,92). This effect is highlighted by the large number of reports concerning the production of fetal-specific substances by tumours e.g. α-fetoprotein, carcinoembryonic antigen etc. Evidence is obtained mainly by immunological cross-reaction studies i.e. antibodies raised to tumour antigens will also react with fetal cell products (1,2,3,4,19,22,37,75,76,79). The achievement (in mice) of a degree of immunization against oncogenesis using irradiated fetal cells as immunogens (1,18,22,97) further indicates a relationship between fetal and tumour cells.

If neoplasia does cause cells to acquire an earlier competence, then the non-random pattern of ectopic hormone production may be determined by the embryological origin of the cells concerned (8,40,55). This factor, in addition to the morphology and histochemistry of the tumours involved, has been studied intensively (12,24,40,48,50,55, 56,60,66,63,93,94) and the most important development has been the formulation of the APUD concept by Pearse (55,56,57,58,60).

The APUD cell series comprises cells found chiefly in the foregut and its derivatives i.e. pancreas, bronchi, C-cells of thyroid etc., and Pearse grouped them together initially because of the cytochemical and ultrastructural characteristics they had in common (see Table 1). Of particular significance is the capacity of these cells to take up amine precursors (L-DOPA, 5-Hydroxytryptophan)

and decarboxylate them to produce amines. This characteristic, which resembles the process in nerve terminals, gave these cells their mnemonic name (Amine Precursor Uptake and Decarboxylation). Apart from the properties listed in Table 1., a large number of the proposed APUD series are polypeptide-secreting endocrine glands (see Table 2.(

Pearse postulates that APUD cells are neuroectodermal in origin (55,56,57) and, indeed, evidence obtained using the APUD-FIF (APUD formaldehyde induced fluorescence) method (56) to trace the migration of APUD cells from the neural crest (cells with APUD characteristics can be seen here at 72hrs in the chick embryo) supports Pearse's hypothesis. (50,56,57,58) It has been suggested (86) that the APUD cell is, therefore, a pluripotential stem cell, in respect of both amine-handling and polypeptide hormone synthesis, which migrates from the neural crest to endoderm and its derivatives to form the polypeptide-secreting endocrine glands. Despite some evidence,from partial neural crest ablation studies , which indicates that neural crest cells are developmentally labile (96), ther is no positive proof of the APUD cells being pluripotential. What does seem to support this idea is the way in which the APUD concept provides an explanation for the pattern of ectopic hormone secretion. Many of the tumours associated with ectopic hormone production are derived from APUD cells (40,86 and Table 3.) and this could be explained by their common embryological origin. If, as suggested, APUD cells are pluripotent stem cellsw which differentiate into one or other of the peptide endocrine glands, then dedifferentiation in neoplasia would allow any member of the APUD series to synthesize and secrete a variable selection of the whole series of polypeptide hormones (86,91) e.g. ACTH from medullary carcinoma of thyroid or

from an islet-cell carcinoma. It could also account for the secretion of 5-HT frequently associated with these tumours (48).

Levine and Metz (40), in their classification of ectopic hormone producing tumours, have made Group 1 all those tumours derived from APUD cells (see Table 3) and the term APUDOMA has emerged (15, 60,61,91). An APUDOMA can be defined as a tumour derived from an APUD cell and thus neuroectodermal in quality, which is secreting either its normal hormone or one or more of the hormones of the APUD series. (61)

Since the APUD concept was suggested as a possible explanation for the pattern of ectopic hormone production, there has been a steady accumulation of evidence in support of it e.g. the oat-cell, which is reknowned for its association with ectopic hormones, is ultrastructurally very similar to the bronchial carcinoid cell which is derived from the Feyrter or Kultschitsky cell (an APUD cell). The oat-cell is probably an anaplastic derivative of a Feyrter cell (12,34,94). The evidence so far, however, does not account for the occasional reports of production of PTH by tumours possessing APUD characteristics because the parathyroid is presently regarded as being of endodermal origin. Ther are also reports of production of APUD hormones by tumours of known endodermal or mesodermal origin (40,68). These findings may reflect bad documentation or, possibly, an even greater degree of derepression than usual i.e. the association of certain tumours with a hormone is statistical rather than absolute. An alternative explanation (85) is that cell hybridization, which has been shown to occur in malignant tumours (7,38,43,87,88,), may play a part in the genesis of ectopic humoral syndromes. In those cases in which APUD hormones are secreted by tumours of non-APUD origin, the tumour cells might have fused with neuroectodermal cells. This idea seems rather unlikely and has no supporting evidence.

The APUD concept, therefore, appears to account reasonably well for the association of certain tumours with certain hormones. The implication of this, from a genetic point of view, is that, while all cells are genetically totipotent, the genes coding for polypeptide hormone synthesis are more easily derepressed in APUD cells. The molecular mechanism of cell differentiation is incompletely understood (16,20,33,47,75,84) but this seems to suggests that the degree of repression, and, hence, the ease of derepression, is different for different genes, depending on the embryological origin of the cells. (15)

The above discussion is centred around the Group 1 tumours of Levine and Metz (40) i.e. the APUDOMAS, but there is a considerable number of ectopic hormonal syndromes which are associated with non-APUD derived tumours (Group 2). This group is composed of a heterogeneous collection of tumours (see Table 4) of endocrine and non-endocrine cells. They are, however, all derived from either mesoderm or endoderm (40) and it is possible that the shared embryological origin is sufficient to create a pattern in the same way as with the APUD cells. It may be significant that Group 2 tumours are less well differentiated morphologically than Group * 1 tumours and are much more frequently associated with the production of fetal substances (4). This may reflect a greater degree of dedifferentiation which would account for the heterogeneity of this group.

E. SUMMARY AND CONCLUSIONS

The phenomenon of ectopic hormone production by tumours has been discussed and the need for accurate documentation emphasized. Theories on the mechanism of ectopic hormone production require good supporting evidence for the exact cells of origin of the hormone, and such evidence has led to the emergence of the APUD theory for certain ectopic humoral syndromes. This theory, which also has wide implications for polypeptide endocrinology, offers a good explanation for the Group 1 tumours, especially in the light of the evidence for neoplasia producing derepression of the genome and a return to an earlier competence. It was suggested that the behaviour of APUD cells in neoplasia is evidence for differential repression of the genome. More knowledge of the mechanisms of cell differentiation should provide an explanation for how this occurs., as well as increasing understanding of Group 2 tumours.

Ectopic hormone production in tumours, which,at present, is regarded as a relatively rare occurrence, may have wider implications in neoplasia. The behaviour of malignant cells may stem from the production by them of substances normally produced only in the course of development. Our previous knowledge of hormone excess syndromes has allowed us to document the ectopic hormone phenomenon but this may just be one example of a widespread effect.

TABLE 1

a) Cytochemical characteristics of APUD cells

1. Fluorogenic amine content (catecholamine, 5-HT etc.)
2. Amine precursor uptake.
3. Amino-acid decarboxylase.
4. High side-chain carboxyl (masked metachromasia)
5. High non-specific esterase or cholinesterase.
6. High α-glycerophosphatedehydrogenase.
7. Specific immunofluorescence (if known hormone present)

b) Ultrastructural chara cteristics

1. Low rough endoplasmic reticulum (e.r.)
2. High smooth e.r.
3. Electron-dense mitochondria.
4. Prominent microtubules.
5. Large amounts of free ribosomes.
6. Presense of membrane-bound secretiom vesicles.

TABLE 2

THE APUD CELL SERIES

PIUITARY ACTH

PITUITARY MSH

PITUITARY HGH

PANCREATIC ISLET B-CELL (INSULIN)

PANCREATIC ISLET A_2-CELL (GLUGAGON)

PANCREATIC ISLET A_1-CELL (GASTRIN?)

THYROID C-CELL (CALCITONIN)

STOMACH-ARGYROPHIL CELL (GASTRIN)

STOMACH-ENTEROCHROMAFFIN CELL (SECRETIN)

INTESTINE-ARGYROPHIL CELL (CHOLECYSTOKININ-PANCREOZYMIN)

INTESTINE-ENTEROCHROMAFFIN CELL (SECRETIN,GLUCAGON)

CAROTID BODY TYPE I CELL(?)

LUNG ENDOCRINE CELL (FEYRTER) (?)

See ref 15 and61.

DOCUMENTATION OF HORMONE SECRETION BY GROUP II TUMORS

	Parathormone	Erythropoietin	Gonadotropins and/or Human Placental Lactogen	Prolactin	Growth Hormone	Insulin-Like Activity	Renin	Thyrotropin	Other Classified Hormones
Hepatoma and cholangioma	(T)* +† (1, 2)*	(T) (+) (5)	(T) + (4, 5)			(S) P‡ (5)			ACTH (P) serotonin (P)
Wilms' tumor		(T) (+) (5)				P	(T) + (5)		
Hypernephroma	(T) + (1, 4)	(T) (+) (5)		(S) + (2, 4, 5)			(S) + (2, 5)		ACTH (P)
Adrenal cortical tumors	(T) + (4)	(S) P (5)	(U) P (5)			(T) + (5)			catecholamines (P) ACTH (P)
Gonadal tumors (nongerminal)	P		(U) P (5)			P			ACTH (P)
Vascular tumors	P	(T) (+) (5)				P	(T) P (5)		
Connective tissue and mesodermal tumors	P	P				(T) + (5)			
Reticuloendothelial tumors	(T) + (4)		(T) + (4)			P			vasopressin (P)
Lung tumors (other than oat cell)	(T) + (4)	P	(T) + (1, 3, 4)	(S) P (4,5)	(T) (+) (3,4)	(T) + (5)		(T) + (4, 5)	vasopressin (P) serotonin (P) ACTH (P)
Gastrointestinal tumors	(T) + (4)	P	(T) P (4)			(T) + (5)			
Melanoma§	P	P	(U) P (5)						gastrin (P) vasopressin (P)
Pheochromocytoma and related tumors¶	P	(T) P (5)	(T) + (4)			(T) + (5)			see TABLE 5

* The best available documentation of hormone secretion is presented according to the following code: Hormone localized in tumor(T), or found in plasma or serum(S), or urine(U). There has been demonstrated: (1) a significant arteriovenous gradient across the tumor bed, (2) secretion of the hormone by tumor cells in tissue or cell culture, or (3) localization of hormone in the tumor by specific histofluorescent technique. The hormone has been identified by (4) radioimmunoassay or by (5) bioassay or physicochemical method.

† +, Definite documentation.

‡ P, probable or possible documentation.

§ Possibly transitional.

¶ Transitional group.

DOCUMENTATION OF HORMONE SECRETION BY GROUP I TUMORS

	Catecholamines	Serotonin	Insulin	Calcitonin	ACTH-Melanocyte-Stimulating Hormone	Vasopressin	Gastrin and/or Glucagon	Secretin	Other Classified Hormones
Oat cell tumor		(T)* +† (1, 3)*	(T) P‡ (4, 5)	(T) P	(T) + (3)	(T) + (2, 4)	(T) P (4)		human chorionic gonadotropin (+) parathormone (+) renin (P)
Foregut carcinoid		(T) + (3)	(T) P (4)	(S) P (4)	(T) + (1, 2, 3)	P	P		
Islet cell and pancreatic duct tumors	P	(T) + (3)	(T) + (3)		(T) + (3)	(T) + (4, 5)	(T) + (4, 5)	(T) + (4, 5)	parathormone (+)
Thyroid medullary carcinoma	P	(T) + (3)		(T) + (2, 3)	(T) + (4, 5)				
Epithelial thymoma				P	(T) + (2, 4)	P			
Pheochromocytoma and related tumors§	(T) + (1, 2, 3)	P	P	P	(T) + (4, 5)		P	P	see TABLE 6

* The best available documentation of hormone secretion is presented according to the following code: Hormone localized in tumor(T), or found in plasma or serum(S), or urine(U). There has been demonstrated: (1) a significant arteriovenous gradient across the tumor bed, (2) secretion of the hormone by tumor cells in tissue or cell culture, or (3) localization of hormone in the tumor by specific histofluorescent technique. The hormone has been identified by (4) radioimmunoassay or by (5) bioassay or physicochemical method.

† +, Definite documentation.

‡ P, probable or possible documentation.

§ Transitional group.

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